

IN THE SPECIFICATION

Please replace the paragraph beginning on line 4 of page 3 with the following:

Since the various restriction enzymes appear to perform similar biological roles, in much the same ways, it might be thought that they would resemble one another closely in amino acid sequence and behavior. Experience shows this not to be true, however. Surprisingly, far from resembling one another, most Type II restriction enzymes appear unique, resembling neither other restriction enzymes nor any other known kind of protein. Type II restriction endonucleases seem to have arisen independently of one another for the most part during evolution, and to have done so hundreds of times, so that today's enzymes represent a heterogeneous collection rather than a discrete family. Some restriction endonucleases act as homodimers, some as monomers, others as heterodimers. Some bind symmetric sequences, others asymmetric sequences; some bind continuous sequences, others discontinuous sequences; some bind unique sequences, others multiple sequences. Some are accompanied by a single methyltransferase, others by two, and yet others by none at all. When two methyltransferases are present, sometimes they are separate proteins, at other times they are fused. The orders and orientations of restriction and modification genes vary, with all possible organizations occurring. Several kinds of methyltransferases exist, some methylating adenines (m6A-MTases), others methylating cytosines at the N-4 position (m4C-MTases),

MTases), or at the 5 position (m5C-MMases MTases). Usually there is no way of predicting, *a priori*, which modifications will block a particular restriction endonuclease, which kind(s) of methyltransferases(s) will accompany that restriction endonuclease in any specific instance, nor what their gene orders or orientations will be.

Please replace the paragraph beginning on line 14 of page 9 with the following paragraph:

Since expression from a medium-copy-number T7 vector in *E. coli* pre-modified with M.*Pst*I did not generate a stable high expression clone, efforts were made to express the *sbfIR* gene in M.*Sbf*I methylated *E. coli* using pLT7K. When the *Sbf*I endonuclease gene was cloned in M.*Sbf*I pre-modified *E. coli*, a stable and over-expressing clone was established. Over-expression of an enzyme is generally intended to mean at least ~~10⁵ug~~ 10⁵ units/g including 10⁶ or ~~10⁷ug~~ 10⁷ units/g. Low expression is less than ~~10³ug~~ 10³ units/g. Low expression levels of a putative cloned restriction endonuclease may result in cleavage profiles of DNA. However, this does not conclusively prove that the desired enzyme has been obtained. For example, the enzyme digest may be partial or incomplete making it unclear whether the products are merely the result of random cleavage.

Please replace the paragraph beginning on line 30 of page 20 with the following paragraph:

Varying units of restriction enzymes *Sau3AI*, *BclI*, *BstYI*, *BglII*, *XbaI* and *NheI* were used to digest 10 ~~mg~~ μ g genomic DNA to achieve complete and limited partial digestion. The digested DNA was purified via phenol-CHCl₃ extraction and isopropanol precipitation. The *Sau3AI*-, *BclI*-, *BstYI*-, and *BglII*-, digested DNAs were ligated to *BamHI*- digested and CIP-treated pUC19 vector containing 2 *SbfI* sites. *XbaI*- and *NheI*-, digested DNAs were ligated to the same vector *XbaI*- digested and CIP treated. Following overnight ligation, the DNA was used to transform an endA⁻ host (ER2502, ER2683 New England Biolabs' collection (Beverly, MA)), made competent by CaCl₂ method. Approximately 2-5,000 Ap^R transformants were obtained from each library. For each enzyme, colonies were pooled and amplified in 500ml LB + Ap Amp overnight. Plasmid DNA was prepared by CsCl gradient purification, resulting in a primary library.

Please replace the paragraph beginning on line 18 of page 21 with the following paragraph:

The primary plasmid DNA library (1 ~~mg~~ μ g DNA) was challenged by digestion with ~30 units of *SbfI* at 37°C for 1 hour. The digested DNA was transferred into ER2502 or ER2683 by transformation, resulting in ~750 Ap^R survivors from all libraries. Plasmid DNA from ~120 survivors was prepared by the Compass Mini Plasmid Kit method, followed by *SbfI* digestion. No resistant clones were found from any of the libraries. Some remaining survivors (*Sau3AI*, *BstYI*, *XbaI* and *NheI*) were also pooled separately to form secondary libraries, challenged with *SbfI* a second

time, followed by the same survivor Plasmid DNA purification, again no resistant clones were found.

Please replace the paragraph beginning on line 10 of page 24 with the following paragraph:

As a positive control, converging primers 3A and 5C, were used on *SbfI* chromosomal DNA. For the inverse PCR, genomic DNA was individually digested with *BstBI*, *BstUI*, *DraI*, *HincII*, *HpyCH4IV*, *RsaI* and *ScaI*. The digestions were inactivated at 65°C for 20 min. Self-ligation was set up at a low DNA concentration at 2 ~~mg/ml~~ µg/ml overnight at 17°C. The resulting circular DNA preps were used as the templates for inverse PCR. PCR conditions were 95°C for 5 min, 1 cycle; 95°C for 1 min, 62°C for 1 min, 72°C for 2 min for 25 cycles. Converging primers 3A and 5C generated the *sbfIR* ~400 bp control fragment. Inverse PCR products were found in the *HincII* and *HpyCH4IV* templates. The PCR products were gel-purified, phenol/CH₃Cl-extracted and isopropanol-precipitated. Immediately downstream of the 3B primer within the *sbfIR* N-terminus is an *ApoI* site. This *ApoI* site was used to digest the inverse PCR products at this site, followed by overnight ligation into *EcoRI*- and *HincII*- digested pUC19. The ligated DNA was transferred into ER2502 and ER2683 by transformation. Plasmids were identified that contained the inverse PCR fragment and sequenced directly with pUC19 universal primers 1233 and 1224. Using this DNA sequence, another direct PCR was done with 3B and a newly designed converging primer Sb-3 having the following sequence:

Please replace the paragraph beginning on line 15 of page 30 with the following paragraph:

The genomic DNA was digested with *HincII* in appropriate restriction buffer and inactivated at 65°C for 20 min. Self-ligation was set up at a low DNA concentration at ~~2 mg/ml~~ µg/ml overnight at 17°C. The circular DNA product was used as the template for inverse PCR. PCR conditions were 94°C for 5 min, 1 cycle; 94°C for 30 sec, 62°C for 1 min, 72°C for 1 min for 25 cycles. The PCR fragment was gel purified from an agarose gel, phenol/CH₃Cl-extracted and isopropanol precipitated. The resuspended PCR fragment was blunt-end ligated at 17°C overnight into pCAB16 digested at the *BsaAI* site followed by transformation into ER2502 *E. coli* cells. Plasmid DNA was purified from twelve colonies. Ten appeared to contain the PCR DNA fragment. Four clones were sequenced directly with the following primers:

Please replace the paragraph beginning on line 23 of page 33 with the following paragraph:

DNA sequence showed an intact *sbfIR* in the opposite orientation to Plac and *mspIR*. The pCAB16-*sbfIR* #1 and #7 clones contained correct DNA sequence for *sbfIR*, so an attempt was made to subclone the *SbfI* endonuclease gene from pCAB16-*sbfIR* #1 and #7 into pLT7K, and then transform into ER2744 [pACYC184-PstIM]. Using the flanking *BamHI* and *XhoI* sites designed within the PCR primers, 10 µg of each pCAB16-*sbfIR*

plasmid was digested with *Bam*HI and *Xho*I at 37°C for 2 hours and the *sbf*IR fragment were gel purified from a agarose gel, phenol-CH₃Cl extracted and isopropanol precipitated. The resuspended PCR fragment was ~~was~~ ligated at 17°C overnight into pLT7K with compatible ends, followed by transformation into ER2744 [pACYC184-PstIM] and plated on Ap^R Cm^R plates at 37°C. Plasmid DNA's were purified from 18 colonies (9 each from gel pure pCAB16-*sbf*IR #1 and #7, respectfully), 6 clones were found to carry the PCR insert. pLT7K-S*bf*IR #5, #12 and #14 were inoculated into pre-warmed 10 ml cultures containing LB+Ap^R Cm^R and grown at 37°C overnight without shaking. 2 ml of the overnight cultures were diluted in pre-warmed 50 ml cultures containing LB+Ap^R Cm^R and grown at 37°C to an OD₅₉₀ of between 0.8 and 1.0, IPTG was to added to 85 mg/L and induced at 30°C for ~2 hours. Cells were harvested and lysed by sonication. Clarified cell lysates were assayed for *Sbf*I activity on I DNA. The extracts generated partial *Sbf*I digestion pattern. pLT7K-S*bf*IR #12 was sequenced with the following primers:

Please replace the paragraph beginning on line 29 of page 36 with the following paragraph:

The plasmid pACYC184-S*bf*IM #7 was transferred into ER2848 to premodify *E. coli*. Competent cells were made by CaCl₂ method and the final strategy was employed in which the *sbf*IM gene was expressed from a low-copy-number plasmid [pACYC184-S*bf*IM] and the endonuclease gene from [pLT7K-S*bf*IR]. Isolate pLT7K-S*bf*IR #12 was transferred into ER2848 [pACYC184-S*bf*IM] and plated on Ap^R Cm^R plates at 37°C

overnight. Two individual colonies were inoculated into 10 ml LB+Ap^R Cm^R and grown at 37°C overnight. 1 ml of each overnight culture was inoculated into 50 ml of LB+Ap^R Cm^R and grown at 37°C to OD₅₉₀ 0.8 to 1.0, then the culture temperature was then lowered to 30°C, followed by IPTG (85 mg/L) induction at 30°C for 2 hours to overnight. Both individual clones expressed R.*SbfI* at more $\sim 10^5$ ~~u/g per gram~~ units/g of wet *E. coli* cells (Figure 8).